slow compared to the docking reaction, so actual observation of single docking events in real time was extremely rare. Here, we present a new method of tethering vesicles that is orthogonal to the DNA-mediated docking and fusion reactions. Vesicles are covalently attached to the supported lipid bilayer by a DNA-templated click reaction, allowing simultaneous deposition of cognate vesicle populations displaying complementary DNA. This results in well-mixed populations of tethered vesicles, which diffuse randomly in two dimensions. Upon raising the salt concentration, tethered vesicles can be triggered to interact with each other via DNA hybridization. An additional parameter probed with this strategy is the location of the templating DNA-lipid, which can be anchored in the vesicle or in the supporting bilayer. The mobility, as measured by single particle tracking, of vesicles anchored by two lipids is approximately 1.6-fold slower than that of vesicles anchored only with a single lipid.

* Chan, Y-H M *et al*. 2007. *Proc Natl Acad Sci USA* 104:18913.

3513-Pos

Role of Curvature in PEG-Mediated Fusion Between Highly Curved and Un-Curved Membranes

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During neurotransmitter release, curved synaptic vesicles fuse with un-curved pre-synaptic plasma membrane, leading to the merger of two lipid bilayers and the release of neurotransmitters. Our previous efforts to model this system have employed two populations of highly curved vesicles, while others have used two populations of vesicles having ill-defined but lower curvature. Here we examine poly ethylene glycol (PEG)-triggered fusion of highly curved (SUV) with relatively un-curved (LUV) vesicles, composed of a mixture of DOPC/DOPE/sphingomeylin/DOPS/cholesterol (32/25/15/8/20), which closely models the lipid composition of synaptic vesicles. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to 3- or 4-state sequential models (Weinreb, Biophys. J., 2007), from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state. As expected, un-curved LUV-LUV fusion was barely detectable, while highly curved SUV-SUV fusion was reasonably efficient, saturating at ~50% LM and ~40% CM. Remarkably, SUV-LUV fusion was decidedly more efficient, saturating at nearly 100% LM and CM. Analysis of the fusion kinetics at different temperatures (17°-42°C) revealed complex activation thermodynamics. The rate of the first fusion intermediate formation is decidedly faster in the highly curved (stressed) SUV-SUV system than in the mixed system, with increasing temperature shifting the probability of CM toward earlier steps; whereas the probability of CM in SUV-LUV fusion shifts towards fusion pore. This suggests that mismatched curvature promotes more efficient and productive fusion events.

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3514-Pos

VSV Trans-Membrane Domain Promotes Content Mixing to occur Early in the Fusion Process

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Polyethylene glycol (PEG)-mediated fusion of 25 nm vesicles was studied at different temperatures in the presence of the trans-membrane domain (TMD) of the G protein (fusion protein) of vesicular stomatitis virus (VSV). Vesicles were composed of dioleyol-phosphatidylcholine (DOPC), dioleyol-phosphatidylethanolamine (DOPE), bovine brain sphingomyelin (SM), and cholesterol (CH) in a molar ratio of 35:30:15:20. Kinetic parameters of the fusion process were determined by fitting lipid mixing (LM), content mixing (CM), and content leakage (L) time courses globally to a three state sequential model that allowed for leakage from the final fusion pore state. This yielded the rate constants for conversion between different states as well as the probabilities of the occurrence of LM and CM in each state. The TMD enhanced the initial rate of lipid mixing and content mixing in agreement with our previous report (Biochemistry, 2002, 14925). This resulted partly from enhanced extent of CM, although the extent of LM remained unaltered. In addition, the rate of intermediate formation was increased slightly, although, the principal effect of TMD was to increase the probability of CM in the initial intermediate at the expense of the final step of the process. This suggests that the presence of TMD enhanced flickering pore formation, leading to an increase in CM early in the process. Supported by NIGMS grant 32707 to BRL.

3515-Pos

Effect of HIV Gp41 Fusion Peptide and its Cross-Linked Oligomers in Membrane Fusion

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Virus-cell membrane fusion is an essential and important step for the infectious entry of a virus into a host cell. During HIV infection gp41 fusion protein plays an essential role in membrane fusion. Recently, it has been reported that a trimeric form of gp41 fusion peptide is optimally catalytically efficient for lipid mixing between lipid vesicles. We have characterized the effect of different oligomers (monomer, dimer, trimer and tetramer) of gp41 fusion peptide on the entire process of 23 nm vesicle fusion. Time courses of lipid mixing, content mixing, and content leakage were determined for control vesicles as well as for vesicles in presence of different oligomers. We were able to describe data for all systems using a one-intermediate leaky fusion model, and obtained thereby rate constants of the three steps in this model. An increase in the extent of lipid mixing was observed in presence of the different chemically cross-linked oligomers, as well as a very small amount of content mixing (~2%). All species induced substantial content leakage, with the trimer and tetramer being most membrane disruptive. The rate of lipid mixing and content mixing increased in the order of monomer $<$dimer $<$trimer $\approx$ tetramer. The oligomers affected fusion induced by 5% poly(ethylene) glycol (PEG) in a completely opposite fashion (i.e., they inhibited both). Quasi-elastic light scattering (QELS) and static 900 light scattering data in presence of different oligomers suggested formation of larger aggregates in the presence of all oligomers, and the size and polydispersity of the aggregates followed the same trend seen for lipid mixing. Supported by USPHS grant GM32707 to BRL.

3516-Pos

Fusogenic Activity of PLA_2-IIA and SMase in PEG-Mediated Membrane Fusion

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Phospholipase A_2-IIA (PLA_2-IIA) and sphingomyelinase (SMase) have been shown to induce fusion of membranes both *in vivo* and *in vitro*. The fusogenic potential of these two enzymes has been observed, for example, during neurotransmitter release. It is also believed that the products of hydrolysis of these two enzymes induce a reduction in the free energy cost of the fusion event. Assessment of the biophysical mechanisms by which these enzymes propel membrane fusion may provide relevant information for a variety of biological scenarios including neurotransmitter release. Poly(ethylene glycol)-(PEG-) mediated fusion of small unilamellar vesicles (SUVs) was studied in the presence of PLA_2-IIA and SMase at varying temperatures between 17C and 40C. Lipid composition was dioleoylphosphatidylcholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), porcine brain sphingomyelin (SM), and cholesterol (CH) in a ratio of 35:30:15:20. Content mixing (CM), lipid mixing (LM) and leakage (L) time courses were obtained and globally fitted to a 3 or 4-state sequential vesicle fusion model introduced by Weinberg and Lentz (Weinberg and Lentz, Biophys. J., 2007, 92; 4012). Based on the global fits, rate constants for the transitions between states, and LM, CM and L occurrence probabilities are obtained. We observe that both LM and CM are enhanced by enzyme activity in SUVs. In addition, the exogenous addition of hydrolysis products also promotes LM and CM in the absence of the enzymes. A careful study of the temperature dependent plots is used to explore alterations in the energy barriers between intermediate states. These findings may shed light into the biological mechanisms wherein specific vesicle lipid structure and enzyme catalysis become interrelated to induce membrane fusion.

3517-Pos

Membrane Fusion is Spatially Controlled by Modification of Phosphoinositides

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Nuclear envelope assembly is an essential event in cell cycle but its mechanism and regulation remain mostly unknown. Using a cell-free system derived from sea urchin gametes we report that nuclear envelope formation involves the fusion of membrane vesicles highly enriched in phosphoinositides via the production of a fusogenic lipid, the diacylglycerol. By performing time course fluorescence lifetime imagery, we measured the kinetic of this process and demonstrate that nuclear envelope assembly is polarised. It is initiated at the poles of the nucleus where the nuclear envelope remnants are located. This study provides a mechanism for temporal control of NE assembly and offers an explanation for how such a process of membrane fusion can be spatially regulated.